QUANTITATIVE WOOD ANATOMY CERTAIN GEOMETRICAL-STATISTICAL RELATIONSHIPS

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ABSTRACT

A method for the quantitative characterization of wood structure is described. It is based on the principles and techniques of stereology developed first by material scientists. The method has its major advantage in that it does not require direct measurement of structural elements, only counts of points and intersections using a grid system superimposed on the images of microscopic structures. From the basic counts, size distribution parameters are calculated based on formulae derived from geometrical-statistical relationships. The relationships are presented in the form of equations for the calculation of size distribution parameters from simple counts.

Keywords: Wood anatomy, quantitative anatomy, stereology, cell size, size distributions.

INTRODUCTION

Wood is a product of a complex biological system, the tree, and as such it is a highly variable material. Its anatomical structure and properties vary from species to species, from tree to tree of the same species, and even from one part of a single tree to the other. It has been shown by many investigators that property differences are closely related to structure at both macroscopic and microscopic levels. Thus utility of a piece of wood for a specific application is dependent upon its properties which, in turn, are influenced by structure.

The presently accepted practice of wood characterization is based on anatomical structure that serves as the principal method of identification (Panshin and deZeeuw 1980). Structural characteristics considered in the identification keys seldom give a quantitative assessment of the anatomical elements. Furthermore, successful use of the identification keys requires special skills and long training to achieve the desired consistency in making a series of subjective two-way decisions as to the size, distribution, and shape of the anatomical elements. Thus conventional identification methods often fail to give numerical assessments of wood structure needed for predicting properties and performance of wood in various applications or processes.

Since wood anatomy and identification involve the recognition of shapes, sizes, and distribution of elements or features, it should greatly benefit from a quantitative treatment of these features. It is therefore surprising that wood anatomy has only occasionally been subjected to the methods of quantitative microscopy. In the meantime, several branches of science have developed sophisticated mathematical methods to quantify the structure of their respective materials. For example, notable advances have occurred in medical science (Weibel and Bolender 1973), where changes in cellular structure have been related to various illnesses. Metallurgists have for some time been interested in particle size and size distribution for crystals and phases in alloys (DeHoff and Rhines 1968; Gladman and

Woodhead 1960; Hilliard 1966). From the initially simple methods, highly instrumented techniques have developed to provide data for quantitative microscopy.

Conventional wood anatomy distinguishes two types of elements with respect to the direction of the tissues they are a part of in the tree. On this basis, all woods have longitudinal and transverse elements. The longitudinal elements may be vessels, various types of fibers, tracheids, or parenchyma cells. The transverse tissues, the wood rays, may contain tracheids or parenchyma elements or both. Relative size and size-distribution within the growth increments, or annual rings, of these elements are used for identification. However, there is an almost infinite variety of possible arrangements of these elements in the three mutually perpendicular planes with respect to the tree stem that are used in descriptive anatomy for identification. Since the size, distribution, and size-distribution of the elements in highly complex structures such as wood are difficult for the human eye to recognize, minute features or sculpturings on the elements are often used as secondary features for identication.

It is often desirable to tabulate the distinguishing features and their presence or absence in the various species in order to provide an aid to the microscopist. Certain distribution patterns have to be judged subjectively according to these tables to be "gradual" or "abrupt," "diffuse-porous" or "semi-ring porous," "up to 4 seriate," "widest more than 10," "chains," "nested," "multiples," etc. (Panshin and deZeeuw 1980). All these distribution patterns are quantifiable.

Subjective decisions regarding the question of the presence or absence of some elements may be answered with either yes or no, or possibly not at all with any certainty. This points out the need for a statistical approach. With proper sampling and statistical treatment, the probability of the presence of a certain feature in a particular wood may be calculated. A quantitative approach should also provide the necessary basis for the calculation of probabilities. Although conventional descriptive anatomy can be used for distinguishing between species of wood, it does not provide any information for the prediction of properties. Description in qualitative terms is a one-way street providing basically one result, a possible identification, with certainty related only to the knowledge and experience of the anatomist.

The history of wood identification started with the advent of plant anatomy in the late 17th century (Kisser 1967). During the 300-year history, only a few attempts at quantifying anatomical structure of wood are documented in the literature. Most of the work was done on coniferous woods with simple microscopic structure (Denyer et al. 1966; Scallan and Green 1973; Smith 1967). Ladell (1959) and later Kellogg and Ifju (1962) did attempt quantitative assessments of wood structure by applying point countings to microscopic images of some angiosperm woods that had complex anatomical structures. These attempts, however, did not result in the calculation of all the important parameters necessary for the complete quantitative characterization of the structure studied. Steele et al. (1976), Ifju and Chimelo (1978) and Ifju et al. (1978) showed that the principles and techniques of stereology may be used as valuable tools for the quantitative characterization of the microstructure of wood.

The objectives of this paper are to introduce a method for the quantitative characterization of wood microstructure based on simple countings rather than

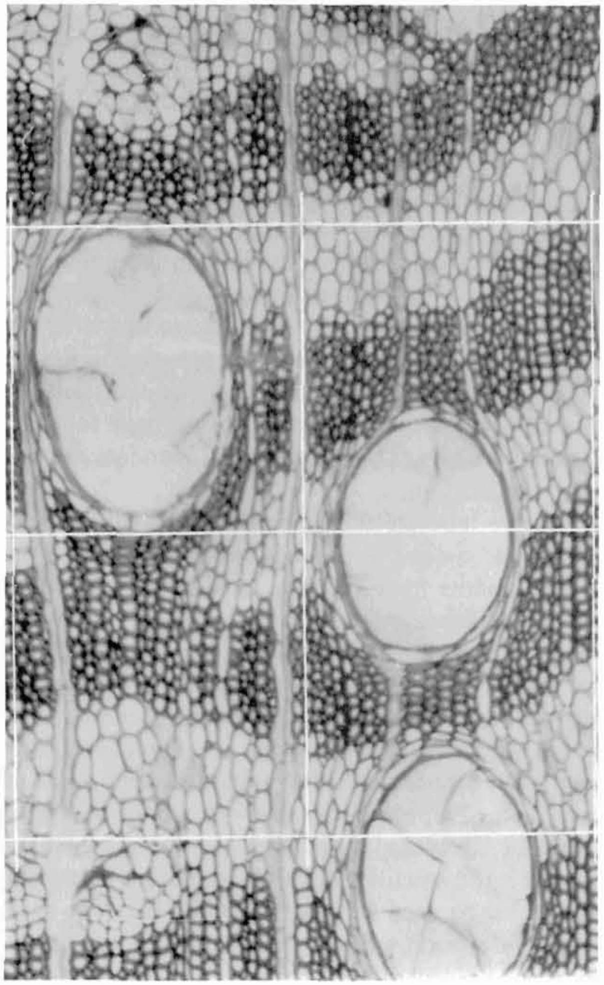

FIG. 1. A nine-point grid superimposed over the transverse section of a diffuse porous wood. Note that the grid lines are oriented parallel and perpendicular to the rays.

on direct measurements and to develop certain geometrical-statistical relationships thereof.

THE STEREOLOGICAL BASIS

The quantitative characterization of microstructure involves the application of the geometrical-statistical techniques and equations that relate measurements upon two-dimensional sections to three-dimensional structural quantities. This type of quantitative analysis is called "stereology" (Underwood 1970) or "quantitative microscopy" (DeHoff and Rhines 1968). The statistical sampling of the microstructure involves measurements upon section images formed either by reflected or transmitted radiation. Measured quantities such as point fractions, intercept and feature counts are then related to structural quantities such as volume fraction (or volume percentage), boundary or surface area per unit volume, mean chord intercept, mean chord spacing of micro-elements, diameters, etc.

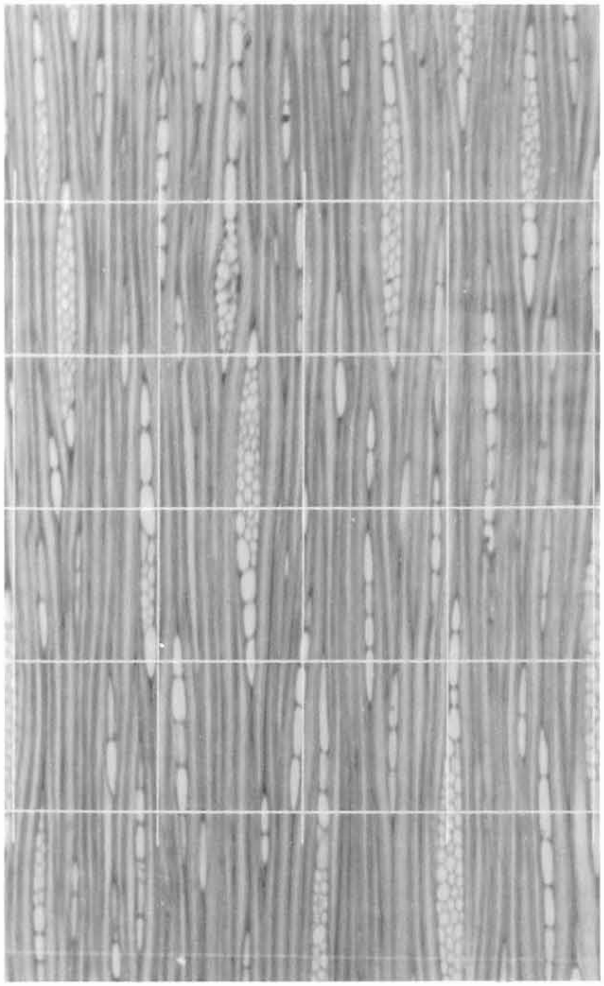

FIG. 2. A twenty-five-point grid superimposed over a tangential-longitudinal wood section. The grid lines are oriented perpendicular and parallel to the long axis of the rays.

The fundamental parameters of structural composition as described by Weibel and Bolender (1973) are the volume density, V_V, surface or boundary density, S_V, numerical density, N_V, and the mean size. The basic stereological counting measurements that may be applied to characterizing wood microstructure are described below.

Point fraction or point counting (P_P)

Point counting (P_P) is one of the simplest operations of quantitative structural analysis. The term refers to the number of test points that are coincident with a particular structure divided by the total number of test points.

The relative amount of each microconstituent in wood can be determined using the point counting technique. These test points could be the intersections of a test grid or the end points of short test lines or random points on a grid as shown in Figs. 1 and 2. Figure 1 shows a 9-point grid of lines whose intersections represent the test points. The application of this grid to cross-sectional images allows the number and, consequently, the fraction of points lying over each type of microconstituent to be considered. Figure 2 is a 25-point grid applied to tangential-section images allowing calculation of the fraction of points lying over the rays. Each point counting represents a random statistic which is an unbiased estimator of the area fraction (A_A) and volume fraction (V_V) (Underwood 1970). The average of a set of random applications of a point counting grid can provide quantitative data concerning the amount of each element with a desired statistical confidence. The relative amount of each cell type or feature can be measured to within any specific statistical accuracy.

The stereological equation that relates the average point fraction ($\bar{P}_P$), lineal fraction ($\bar{L}_L$), area fraction ($\bar{A}_A$), and volume fraction ($\bar{V}_V$), is given by:

$$\bar{V}_V \doteq \bar{A}_A \doteq \bar{L}_L \doteq \bar{P}_P \quad (1)$$

where $\bar{P}_P$ is the average of several randomly applied point fractions (Underwood 1970).

Intersection counting (P_L)

Another important measurement frequently required in quantitative stereology is P_L, the number of point intersections with boundaries generated per unit length of a test line. The procedure involves superposition of directed line segments upon the microscopic section images. A count of the number of times that the line segments intersect the cell boundary when divided by the actual segment length will give the number of intersections per unit length. A linear or circular test array may be applied randomly or placed systematically over the entire microstructure until a sufficient number of intersections have been counted. The actual total grid length (L) depends on the magnification of the microstructure, but its value can be determined at a standard magnification. The P_L measurement is an unbiased statistical estimator of half the surface (cell boundary) area per unit volume (DeHoff and Rhines 1968). As in Fig. 1, the line segments can be applied parallel and perpendicular to the rays. In Fig. 2, the line segments are applied parallel and perpendicular to the tangential images of ray sections.

Intercept counting (N_L)

N_L is another measurement similar to P_L and is defined as the number of features of microstructure intercepted per unit length of the test lines.

The stereological equations that relate the intersection counting (P_L), the intercept counting (N_L), to the surface area (S_V) of cells are as follows:

$$\bar{S}_V \doteq 2\bar{P}_L \quad \text{or} \quad \bar{S}_V \doteq 4\bar{N}_L \quad (2)$$

$$\text{since } \bar{P}_L = 2\bar{N}_L \quad \text{or} \quad \bar{N}_L = \frac{\bar{P}_L}{2} \quad (3)$$

where $\bar{S}_V$ is the mean boundary area per unit volume, which is the area in the test volume occupied by the cell, or surface density, and where $\bar{P}_L$ is the average of the intersection counts.

Feature counting (N_A)

N_A is the number of objects or features in a certain area of the microstructure. Determination of the quantity ($\bar{N}_A$) also allows the average area ($\bar{A}$) of the cells to be calculated using the following formula:

$$\bar{A} = \frac{\bar{A}_A}{\bar{N}_A} \doteq \frac{\bar{P}_P}{\bar{N}_A}, \quad \text{thus,} \quad \bar{A} \doteq \frac{\bar{P}_P}{\bar{N}_A} \tag{4}$$

Calculated size parameters

The intersection count (P_L) may be related to the length of the perimeter that the wood cells form on a section (Underwood 1970). This relationship can be expressed as:

$$\bar{L}_A \doteq \left(\frac{\pi}{2}\right)\bar{P}_L \tag{5}$$

where $\bar{L}_A$ is the total perimeter length per unit area of observations and $\bar{P}_L$ is the average point intersections per unit length of test line. In a non-space-filling system, where there is more than one type of element or cell, as in angiosperm woods (e.g., vessels and fibers, etc.), or when a particular element does not completely fill the whole area,

$$\bar{P}_L = 2\bar{N}_L \quad \text{or} \quad \bar{N}_L = \frac{P_L}{2} \tag{3}$$

as shown earlier. Thus, the average perimeter ($\bar{L}$) for circular cells can be estimated if the number of cells per unit area ($\bar{N}_A$) is determined. This equation can be expressed as:

$$\bar{L} = \frac{\bar{L}_A}{\bar{N}_A} = \frac{\frac{\pi}{2}\bar{P}_L}{\bar{N}_A} = \frac{\pi}{2}\frac{\bar{P}_L}{\bar{N}_A} = \pi\bar{d} \qquad \text{(Underwood 1970)} \tag{6}$$

where $\bar{d}$ is the average cell diameter of a circular element and $\bar{L}$ is the average perimeter length for that cell. From Eq. (6) it may be seen that the mean diameter ($\bar{d}$) of anatomical elements of circular cross sections may be calculated using the simple countings of P_L and N_A.

The mean cell size can be statistically estimated from Eq. (6) or by defining the average chord intercept length of a random test line with an element as:

$$\bar{\lambda}_{MCI} = \frac{1}{N}\left(\sum_{i=1}^{N} L_i\right) \tag{7}$$

where L_i represents a set of N chords measured on a section. Dividing both numerator and denominator by the total length of lines used to measure the set of N chords, L_i, yields:

$$\bar{\lambda}_{MCI} = \frac{\sum_{i=1}^{N} (L_i/L_{TOT})}{N/L_{TOT}} = \frac{\bar{L}_L}{\bar{N}_L} \tag{8}$$

The value of $\bar{L}_L$ is estimated by $\bar{P}_P$ and the value of $\bar{N}_L$ by $\bar{P}_L/2$, since each chord produces two intersections. Thus the average chord length ($\bar{\lambda}$) is estimated from the point counting ($\bar{P}_P$) (see Eq. (1)) and the intersect counting ($\bar{P}_L$) as:

$$\bar{\lambda}_{MCI} \doteq \frac{2\bar{P}_P}{\bar{P}_L} \qquad \text{(Underwood 1970)} \tag{9}$$

This quantity represents an orientation-sensitive property, and its value parallel and perpendicular to the direction of secondary growth can be obtained using line segments directed in these particular orientations.

The mean free path or mean free distance (λ_{MFP}) between features may be defined in the same way yielding:

$$\bar{\lambda}_{MFP} = \frac{2(1 - \bar{P}_P)}{\bar{P}_L} \qquad \text{(Underwood 1970)} \tag{10}$$

Size distribution parameters

Transverse sections.—The three counting measurements, the point counting ($\bar{P}_P$), the intersection counting ($\bar{P}_L$), and the feature counting ($\bar{N}_A$) provide a significant amount of quantitative information from section observations. In wood microstructure where the cells are oriented perpendicular to the sections from which the measurements are made, some information about the size distribution of the quasi-circular sections may also be obtained from $\bar{P}_P$, $\bar{P}_L$, and $\bar{N}_A$. This may be done by defining the first two moments of distribution about zero of the section diameters, as (Weibel and Bolender 1973):

$$M_1 = \int_{y=0}^{y=\infty} y \cdot f(y) \cdot dy = \bar{d} \tag{11}$$

$$M_2 = \int_{y=0}^{y=\infty} y^2 \cdot f(y) \cdot dy = \overline{d^2} \tag{12}$$

where $f(y) \cdot dy$ is the probability density function of cell diameters.

The symbol "M_1" is called the first moment of size (diameter) distribution, which is the same as the average diameter. The symbol "M_2" is called the second moment of size (diameter) distribution, which numerically equals the average of the squares of the diameters and, therefore, it may be easily estimated for circular elements as:

$$M_2 = \overline{d^2} = \frac{4}{\pi}\bar{A} \tag{13}$$

But, since

$$\bar{A} = \frac{\bar{A}_A}{\bar{N}_A} \tag{4}$$

and $\bar{A}_A \doteq \bar{P}_P$ according to Eq. (1), then:

$$M_2 = \frac{4}{\pi}\frac{\bar{P}_P}{\bar{N}_A} \tag{14}$$

and from Eq. (6):

$$M_1 = \bar{d} = \frac{\bar{P}_L}{2\bar{N}_A}; \tag{15}$$

Consequently, the sample variance will be:

$$\sigma^2 = M_2 - M_1^2 = \overline{d^2} - \bar{d}^2 = \frac{4}{\pi}\frac{\bar{P}_P}{\bar{N}_A} - \frac{\bar{P}_L^2}{4\bar{N}_A^2} \tag{16}$$

and the coefficient of variation (CV) is then given by:

$$CV = \frac{\sigma}{\bar{d}} = \sqrt{\frac{\sigma^2}{M_1^2}} = \sqrt{\frac{\frac{4}{\pi}\cdot\frac{\bar{P}_P}{\bar{N}_A} - \frac{\bar{P}_L^2}{4\bar{N}_A^2}}{\frac{\bar{P}_L^2}{4\bar{N}_A^2}}} = \sqrt{\frac{16\bar{N}_A\bar{P}_P}{\pi\bar{P}_L^2} - 1}$$

or,

$$CV(\%) = 100\sqrt{\frac{5.093\bar{P}_P\bar{N}_A}{\bar{P}_L^2} - 1} \tag{17}$$

It should be noted that M_2, the second moment of size distribution, is not an orientation-sensitive parameter since neither $\bar{P}_P$ nor $\bar{N}_A$ is dependent on the direction of the grid used for their determination. However, M_1, σ^2, and CV are all sensitive to the orientation of the lines used for the counting measurements of $\bar{P}_L$. Therefore, when the test lines are oriented in the tangential direction, the above parameters may be different from those determined using test lines lying in the radial direction. In certain cases, these differences may be species-specific.

There are several additional stereological parameters that may be derived from simple countings. In this paper, only those given above are shown to characterize the microscopic anatomical elements on cross sections of wood.

Tangential sections.—On tangential sections of wood, the most important characteristics are related to the wood rays. The number of rays per unit area, the size and size distribution parameters, as well as the shapes of rays, have been used for identification in traditional wood anatomy. The relationships between stereological countings on tangential sections and the above-mentioned parameters are presented below. The average height of ray ($RH_{(T)}$) may be calculated from the intersection and feature countings, on the rays, using the tangential section.

$$RH_{(T)} = \frac{\bar{P}_L(\perp)}{2\bar{N}_A} \tag{18}$$

where $\bar{P}_L(\perp)$ is the average number of intersections per unit length of test lines perpendicular to the rays, and $\bar{N}_A$ is the average number of rays per unit area.

Ray width ($RW_{(T)}$) or maximum tangential ray diameter is related to point counting ($\bar{P}_P$) and intersection counting ($\bar{P}_L$) as follows:

$$RW_{(T)} = \frac{C\bar{P}_P}{\bar{P}_L(\perp)} \tag{19}$$

The constant "C" in Eq. (19) is 4.0 if a rhombic shape is assumed for the rays. If elliptical shape is considered, the value of "C" becomes 2.54. The more typical spindle shape for a ray on the tangential section would have a constant between 2.54 and 4.0. For a uniseriate long ray, the constant approaches 2.0.

Shape factors

The shapes in which anatomical elements may occur on transverse or tangential sections may be quantified using the calculated stereological parameters. For example, vessels on cross sections often appear somewhat elongated in the radial direction. The elongated, elliptical shape of the vessels may be determined by calculating the ratio between their tangential and radial sizes. Such vessel shape factor (VSF) may be obtained from two independent stereological measurements as shown below:

$$\mathrm{VSF} = \frac{\bar{d}_{V(T)}}{\bar{d}_{V(R)}} \tag{20}$$

or,

$$\mathrm{VSF} = \frac{\bar{\lambda}_{MCIv(T)}}{\bar{\lambda}_{MCIv(R)}} \tag{21}$$

Since the mean diameter ($\bar{d}$) and the mean chord intercept ($\bar{\lambda}_{MCI}$) are calculated partly from independent and different stereological countings (see Eqs. (9) and (15)), calculating the value of VSF from Eqs. (20) and (21) may serve as a check on the quality of the stereological data. If the values of VSF calculated from two sources do not agree within reasonable limits, the data should be reexamined for possible errors.

Rays as viewed on tangential sections also exhibit various shapes. Some species may have greatly elongated, narrow rays; others may have short and wide forms. Therefore, on the basis of height and width of rays, a shape factor may be calculated (see Eqs. (18) and (19)).

$$\mathrm{RSF}_1 = \frac{\mathrm{RW}_{(T)}}{\mathrm{RH}_{(T)}} = \frac{\dfrac{C\bar{P}_P}{\bar{P}_{L(\perp)}}}{\dfrac{\bar{P}_{L(\perp)}}{2\bar{N}_A}} = \frac{2C\bar{P}_P\bar{N}_A}{\bar{P}_{L}{}^{2}{}_{(\perp)}} \tag{22}$$

Another shape factor (RSF_2) that may be calculated from rays is based on their average width as seen on cross sections (RW_c) and average maximum width ($\mathrm{RW}_{(T)}$) determined on tangential sections. The basis for this shape factor (RSF_2) is that the ray width determined on cross section is an average value between the maximum width and the minimum width because the microtome blade cuts the rays at a random location with respect to height when cross sections are made. This average value will always be smaller than the maximum width ($\mathrm{RW}_{(T)}$). However, how much smaller it will be is dependent upon the shape of the ray. Thus, a shape factor (RSF_2) obtained from dividing $\mathrm{RW}_{(c)}$ by $\mathrm{RW}_{(T)}$ may be used as a possible species specific variable. If:

$$\mathrm{RW}_{(c)} = \frac{2\bar{P}_{P(\text{cross section})}}{P_{L(\perp)}} \tag{23}$$

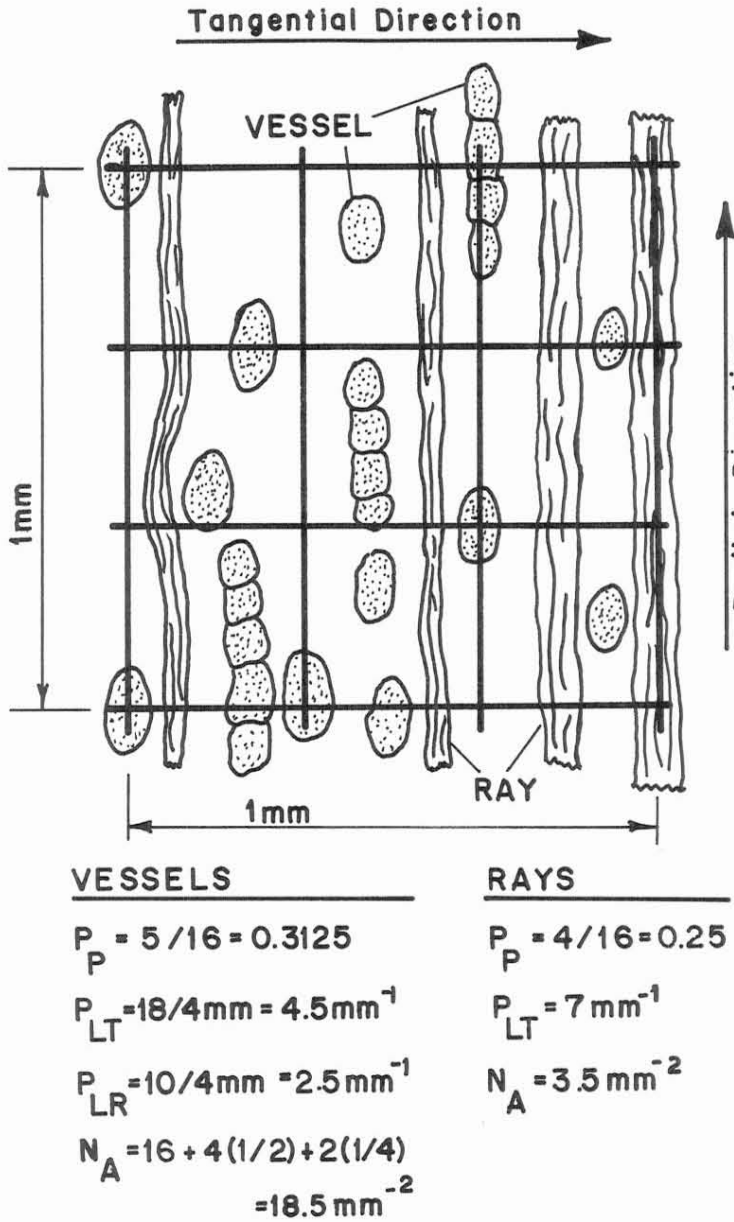

FIG. 3. Example of stereological measurements.

then,

$$\mathrm{RSF}_2 = \frac{\mathrm{RW}_{(c)}}{\mathrm{RW}_{(T)}} = \frac{\dfrac{2\bar{P}_{P(\text{cross section})}}{\bar{P}_{L(T)}}}{\dfrac{C\bar{P}_{P(\text{tangential})}}{\bar{P}_{L(\perp)}}} \tag{24}$$

The relationships shown require a minimum of three types of counting to be performed on microscopic images of the wood samples. These are P_P, P_L, and N_A. The method may involve projecting the microscopic images onto various grids of lines and points, or the insertion of such grids into the eyepiece of a microscope for direct observation of the images. Figure 1 shows one type of grid that may be used. However, in some cases, other grid constructions are more desirable. For example, when the microscopic elements are arranged in a regular pattern, such as the tracheids in conifers, a random distribution of sampling points is more desirable than a regular grid for point and intersection countings.

Example

Figure 3 is a schematic representation of the distribution of vessels and rays on a cross section of a diffuse porous wood. Superimposed on the set of vessels is a sixteen-point grid whose size is equivalent to a 1 mm × 1 mm square on the projected cross section. The intersections of the lines of the grid are the sixteen sampling points. The sampling grid is rotated so that the four vertical and four horizontal lines are oriented in the radial and tangential directions, respectively.

The results of the three counts are also shown in the figure for both vessels and rays. In an experiment, several such counts would have to be made to arrive at representative average values of cell sizes and size distributions. A single set of counts should never be used for size calculations. The number of replications needed to achieve a certain level of confidence at a chosen level of probability has to be determined using one of several possible statistical formulae.

SUMMARY

Quantitative characterization of wood microstructure can be done using the methods of stereology. The numbers so obtained may be useful for relating properties of wood to anatomical structure. It is also conceivable that a computerized probability-based system for wood identification may be developed using the quantitative data obtained with these methods. However, it remains to be determined whether or not these quantitative parameters are sufficiently species-specific to allow development of such a system. It appears that the most likely use of quantitative stereology will be in structure-property relationships especially if instrumental techniques are introduced to collect the basic data.

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